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Synthesis and first in vivo evaluation of new selective high affinity β_1-adrenoceptor radioligands for SPECT based on ICI 89,406

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Abstract—The results of cardiac biopsies suggest that myocardial β_1-adrenoceptor (AR) density is reduced in patients with chronic heart failure, while changes in cardiac β_2-ARs vary. A technique for visualization and quantification of β_1-AR populations rather than total β-AR densities in the human heart would be of great clinical interest. Molecular imaging techniques, either single photon emission computed tomography (SPECT) or positron emission tomography (PET), with appropriate radiopharmaceuticals offer the possibility to assess β-AR density noninvasively in humans, but to date, neither a SPECT nor a PET-radioligand is clinically established for the selective imaging of cardiac β_1-ARs. The aim of this study was to design a high affinity selective β_1-AR radioligand for the noninvasive in vivo imaging of cardiac β_1-AR density in man using SPECT. Based on the well-known selective β_1-AR antagonist, ICI 89,406, both the racemic iodinated target compound **11a** and the (*S*)-enantiomer **15a** were synthesized. Competition studies using the nonselective AR ligand, [^{125}I]iodocyanopindolol ([^{125}I]ICYP), and ventricular membrane preparations from mice showed that **11a** and **15a** possess higher β_1-AR affinities (up to 265-fold) and β_1-AR selectivities (up to 245-fold) than ICI 89,406. Encouraged by these results, the radioiodinated counterparts of racemic **11a** (**11b**: ^{125}I, **11c**: ^{123}I) and (*S*)-configurated **15a** (**15b**: ^{125}I, **15c**: ^{123}I) were synthesized. The target compounds were evaluated in rats. Biodistribution and metabolism studies in rats indicated that there is a specific heart uptake of **11b–c** and especially **15b–c** accompanied by rapid metabolism of the radioligands. Therefore, radioiodinated **11c** and **15c** appeared to be unpromising SPECT-radioligands for assessing β_1-ARs in vivo in the rat. However, the rat may metabolize β-AR ligands more rapidly than other species as demonstrated for (*S*)-[^{11}C]CGP 12177, a radioligand structurally related to **11a–c** and **15a–c**. Therefore further studies in a different animal model will be carried out.

1. Introduction

The heterogeneous family of β-ARs is subdivided into at least three distinct subtypes, the β_1- and β_2-AR[1] and the atypical β_3-AR.[2,3]

β_1-AR agonists increase the heart rate and cardiac contractility and β_2-selective AR agents mainly stimulate bronchodilation and vasodepression.[4] The so-called atypical β_3-ARs are involved in lipolysis.[2] Recently, a putative subtype β_4-AR has been identified in cardiac tissue.[5]

In the normal heart, mainly β_1- and β_2-ARs control the adrenergic functionality of the myocardium. Within the ventricles of the healthy human heart, the β_1/β_2-AR ratio is approximately 80:20.[6,7] In heart disease, however both the β-AR density and the β_1/β_2-AR ratio may change. For example, reduced myocardial β-AR density has been observed in hypertension, heart failure, ischemia and both hypertrophic (HCM) and dilated cardiomyopathies (DCM).[8–13] Studies of biopsies or post mortem material have demonstrated that in a number of heart diseases downregulation of myocardial β_1-AR density is proportionally greater than that of β_2-AR density.[4,14] The failing human heart, however, is often characterized by a selective reduction in β_1-ARs without change in β_2-AR density.[6]

Keywords: 3-Aryloxy-2-propanolamine; ICI 89,406; Selective β_1-adrenoceptor ligand; SPECT-radioligand.
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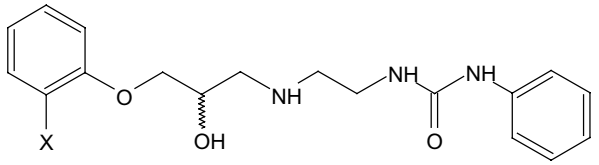
The molecular imaging techniques, single photon emission computed tomography (SPECT) and positron emission tomography (PET), with suitable radioligands may be used to assess β-AR density noninvasively in humans.[15,16] To date, several nonselective β-AR radioligands have been used in cardiac imaging. Radioiodinated derivatives of the β-AR antagonists carazolol and CGP 12177 were synthesized for imaging β-ARs with SPECT.[17–19] (*S*)-[^{11}C]CGP 12177, whose racemate was described by Delforge et al. in 1991,[20] has been used in PET studies to quantify myocardial β-AR density in heart disease[21–23] and recently (*S*)-[^{11}C]CGP 12388 has been presented as a radiochemically more easily accessible radioligand for targeting β-ARs in vivo. [^{18}F]CGP 12388 is also radiochemically available.[24,25]

As the β_1/β_2-AR ratio may change in cardiac disease, however, a procedure for visualization and quantification of β_1-AR population rather than total β-AR densities in the human heart would be of great clinical interest.[7] A few β_1-AR selective radioligands, such as (+/−)-[^{11}C]HX-CH 44,[26] (*S*)-[^{11}C]bisoprolol[27] or [^{11}C]CGP 20712A[28] and its (*S*)-enantiomer [^{11}C]CGP-26505[29] were and are in development for PET, but their clinical use is limited due to high nonspecific binding, rapid metabolism or a tissue uptake that does not reflect binding to β-ARs.[26–29] In summary, no β_1-selective radioligand suitable for the noninvasive assessment of cardiac β_1-AR is clinically established either for SPECT or for PET.

We have chosen ICI 89,406, an early example of a well-designed selective β_1-AR antagonist,[30–32] as the lead compound for the development of high affinity β_1-AR selective ligands suitable for radiolabelling with usual SPECT- or PET-radionuclides. In our initial study we assessed the β_1-AR affinity and selectivity of a number of novel derivatives of ICI 89,406.[33] Of these one, I-ICI-H (Scheme 1) showed high affinity and selectivity for β_1-ARs in myocardial membranes in vitro but high nonspecific binding in vivo.[34] In the present study, we extend these results by inserting a hydrophilic functionality (**11a** and **15a**, Schemes 4a and b), the aim being to reduce the nonspecific binding. In addition to differentiate between the behaviour of the racemate, that can be synthesized in a more cost-effective manner than one particular enantiomer, and the (*S*)-enantiomer the multi-step synthesis and radiolabelling procedures were carried out to yield both the radioiodinated racemic compounds (**11b** and **11c**) and (*S*)-enantiomers (**15b** and **15c**). All synthesized nonradioactive ICI 89,406 derivatives (**8**–**11a** and **12**–**15a**) were assayed in vitro by binding studies, to examine the structure–activity relationships (SAR) between the ligands and β_1-ARs. Biodistribution and metabolism studies were carried out in rats to evaluate the potential of the ^{123}I-labelled compounds **11c** and **15c** as feasible SPECT radioligands.



X: CN ICI 89,406

X: I 2-I-ICI-H

X: ^{125}I 2-[^{125}I]I-ICI-H

Scheme 1. Lead structure ICI 89,406 and its iodinated derivatives 2-I-ICI-H and 2-[^{125}I]I-ICI-H.[33,34]

2. Results

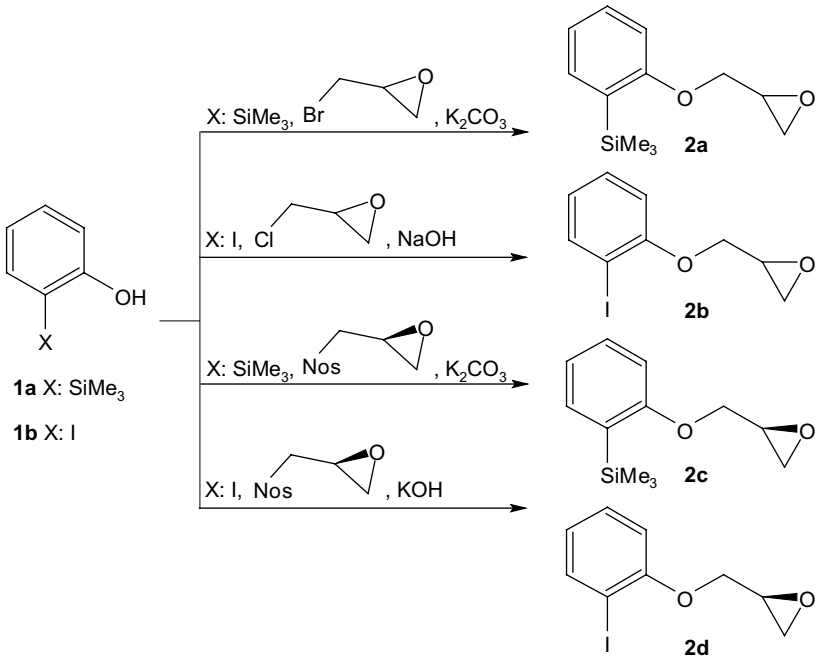
2.1. Chemistry

ICI 89,406 (Scheme 1) is known as a high affinity and selective (75–195 fold) β_1-AR ligand.[34–37] Therefore, the development of a selective β_1-AR radioligand by slight modification of the lead structure is a reasonable approach.

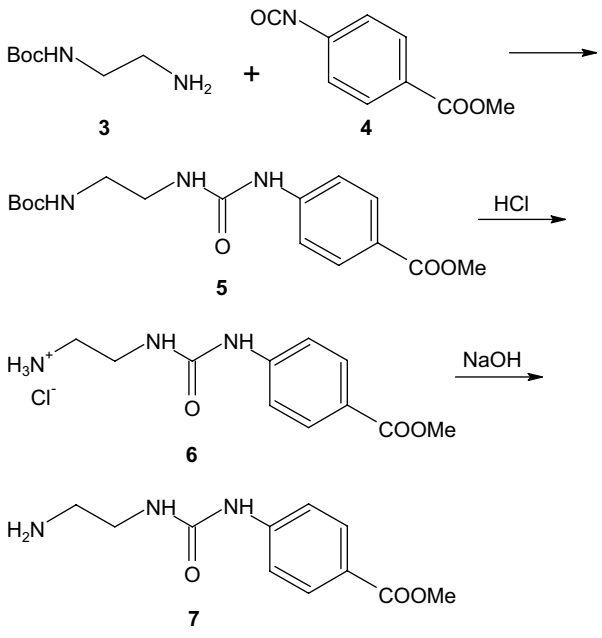
Six pairs of compounds possessing the 3-aryloxy-2-propanolamine core of ICI 89,406 (Schemes 4a and b) were synthesized. Each pair consists of the racemic and the (*S*)-aryloxypropanolamine derivative. Three pairs are chemically identical. Each of them contains a different iodine isotope at the aryloxy unit. The pair **11a** and **15a** represents the nonradioactive version of the ^{125}I-labelled pair **11b** and **15b** that is useful for biodistribution studies. The ^{123}I-labelled pair **11c** and **15c** is also suitable for SPECT and metabolism investigations. The remaining three pairs include the precursors of the radioiodinated compounds (**10**, **14**) and the esterified 3-aryloxy-2-propanolamine derivatives occurring in the synthesis sequence (**8**, **12** and **9**, **13**, respectively). The synthesis of the nonradioactive iodo reference compounds (**11a**, **15a**) and their precursors (**10**, **14**) was realized via a 5–6 step sequence (Schemes 2–4).

The 2-silylated phenol **1a**, that was needed as starting material, was prepared as previously described.[38,39] The 2-substituted phenols (**1a** and **1b**) and the 1,2-epoxy-3-substituted-propane derivatives were converted into the corresponding 3-aryloxy-1,2-epoxy-propanes **2a**–**d** under basic conditions in moderate to excellent chemical yields (44–91%). In the synthesis of the racemic compounds **2a** and **2b** bromide and chloride acted as leaving groups, respectively. The chiral compounds **2c** and **2d** were prepared from (*S*)-glycidyl-3-nitrobenzene sulfonate ((*S*)-glycidyl nosylate) containing the chiral information and a nosylate leaving group (Scheme 2).

The key intermediate amines **6** and **7** were synthesized in 2–3 steps. The condensation of the Boc-protected ethylenediamine **3** and the phenylisocyanate **4**, prepared by a procedure similar to that published elsewhere,[40] yielded the Boc-urea **5** (yield: 88%). After deprotection of **5** under acidic reaction conditions the hydrochloride **6** was isolated (yield: 95%), that was transformed into the free



Scheme 2. Synthesis of the oxiranes **2a**–**d**.



Scheme 3. Synthesis of the amines **6** and **7**.

amine **7** with an equimolar amount of NaOH (yield: 79%) (Scheme 3).

The ring opening of the epoxides **2a**–**d** with the amine **7** or under basic reaction conditions with the hydrochloride **6** gave the 3-aryloxy-2-propanolamine derivatives **8**, **9**, **12** and **13** (yields: 35–55%). The desired precursors (**10** and **14**) and nonradioactive reference compounds (**11a** and **15a**) were made from these esters **8**, **9**, **12** and **13** via saponification (yields: 33–77%, Schemes 4a and b).

2.2. Radiochemistry

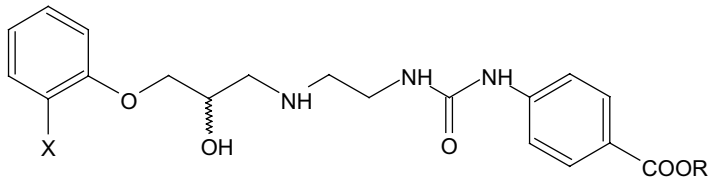
The radioiododesilylation of **10** and **14** was performed under oxidative conditions (NCS) with [^{125}I]NaI or [^{123}I]NaI, respectively. After HPLC-purification the radiolabelled β_1-AR ligands **11b**, **11c**, **15b** and **15c** were obtained. The ^{125}I-labelled compounds **11b** and **15b** gave better radiochemical yields than the ^{123}I-labelled **11c** and **15c** (78 vs 41%, Schemes 4a and b). The radiochemical purity of the compounds was >98% with theoretical specific radioactivities of 0.16 GBq/μg (79.6 GBq/μmol) for **11b** and **15b** and 17.7 GBq/μg (8.77 TBq/μmol) for **11c** and **15c**. Figure 1 shows the HPLC chromatograms of a reaction mixture of **15c** (Fig. 1a), of the corresponding quality control (Fig. 1b) and the reference control of **15c** (Fig. 1c). No impurities could be detected in the HPLC quality control of **15c** while the radioiodinated compound **15c** corresponds to its nonradioactive counterpart **15a** in the HPLC reference chromatogram.

3. Biology

3.1. In vitro studies

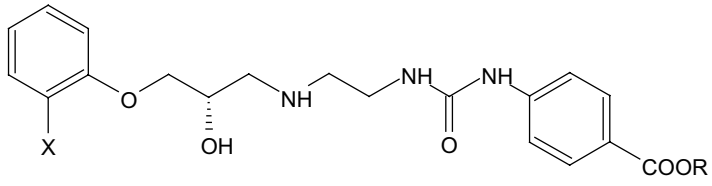
The protein dependence of [^{125}I]iodocyanopindolol ([^{125}I]ICYP) binding to mouse ventricular membrane preparations was investigated in previous work.[34] The specific binding increased linearly with the protein concentration for up to 50 μg/200 μL. Therefore, 15 μg protein per 200 μL were used for the in vitro binding studies. The time course of [^{125}I]ICYP binding to β-ARs was linear for approximately 15 min and reached equilibrium after 60 min of incubation. To determine the maximum binding, assays were carried out for 60 min. The binding of the nonselective β-AR antagonist

(a)



Product No.	Product X	Product R	Educts / Reagents
8	$SiMe_3$	Me	**2a** (Scheme 2), **7** (Scheme 3)
9	I	Me	**2b** (Scheme 2), **6** (Scheme 3), NaOH
10	$SiMe_3$	H	**8**, NaOH
11a	I	H	**9**, NaOH
11b	^{125}I	H	**10**, [^{125}I]NaI, NCS
11c	^{123}I	H	**10**, [^{123}I]NaI, NCS

(b)



Product No.	Product X	Product R	Educts / Reagents
12	$SiMe_3$	Me	**2c** (Scheme 2), **7** (Scheme 3)
13	I	Me	**2d** (Scheme 2), **6** (Scheme 3), NaOH
14	$SiMe_3$	H	**12**, NaOH
15a	I	H	**13**, NaOH
15b	^{125}I	H	**14**, [^{125}I]NaI, NCS
15c	^{123}I	H	**14**, [^{123}I]NaI, NCS

Scheme 4. (a) Synthesis of the racemic β_1-AR ligands **8**–**11c**. (b) Synthesis of the (*S*)-configurated β_1-AR ligands **12**–**15c**.

[^{125}I]ICYP to ventricular membranes was specific, saturable and of high affinity. Scatchard transformation of the saturation data yielded a linear plot with a correlation coefficient greater than 0.95. The dissociation constant (K_D) and the maximum number of binding sites (B_{max}) determined from three experiments for the binding of [^{125}I]ICYP were 32.3 ± 1.9 pM and 38.6 ± 2.9 fmol mg^{-1} protein, respectively.[41] Competition studies were carried out to assess the inhibition of the binding of [^{125}I]ICYP by the β_1-selective compounds **8**–**11a** and **12**–**15a** (Fig. 2a and b). Nonlinear regression analysis using the XMGRACE programme (Linux software) showed that the data of the ligands **8**–**11a** and **12**–**15a** fitted a two-site model significantly better than a one-site model ($F = 2.15$, $p < 0.05$). These compounds show a significantly higher affinity to β_1 than to β_2-ARs (Table 1).

To determine the structure–activity relationships (SAR) between the ligands and β_1-ARs, the high- and low-affinity IC_{50} values of all synthesized and nonradioactive 3-aryloxy-2-propanolamine derivatives **8**–**11a** and **12**–**15a** for the β_1- and β_2-ARs, respectively, were calculated by nonlinear regression analysis of the competition studies using [^{125}I]ICYP and mouse ventricular membrane preparations.

The IC_{50} values were converted into the high- and low-affinity inhibition constants (K_1 for the β_1-ARs and K_2 for the β_2-ARs) by the method of Cheng–Prusoff[42] using the experimentally determined K_D value of [^{125}I]ICYP (32.3 ± 1.9 pM). The ratios of the low- to high-affinity inhibition constants (K_2/K_1) yield the β_1-selectivities of the unlabelled derivatives **8**–**11a** and **12**–**15a** (Table 1). Additionally, the calculated log*P* and log*D* values

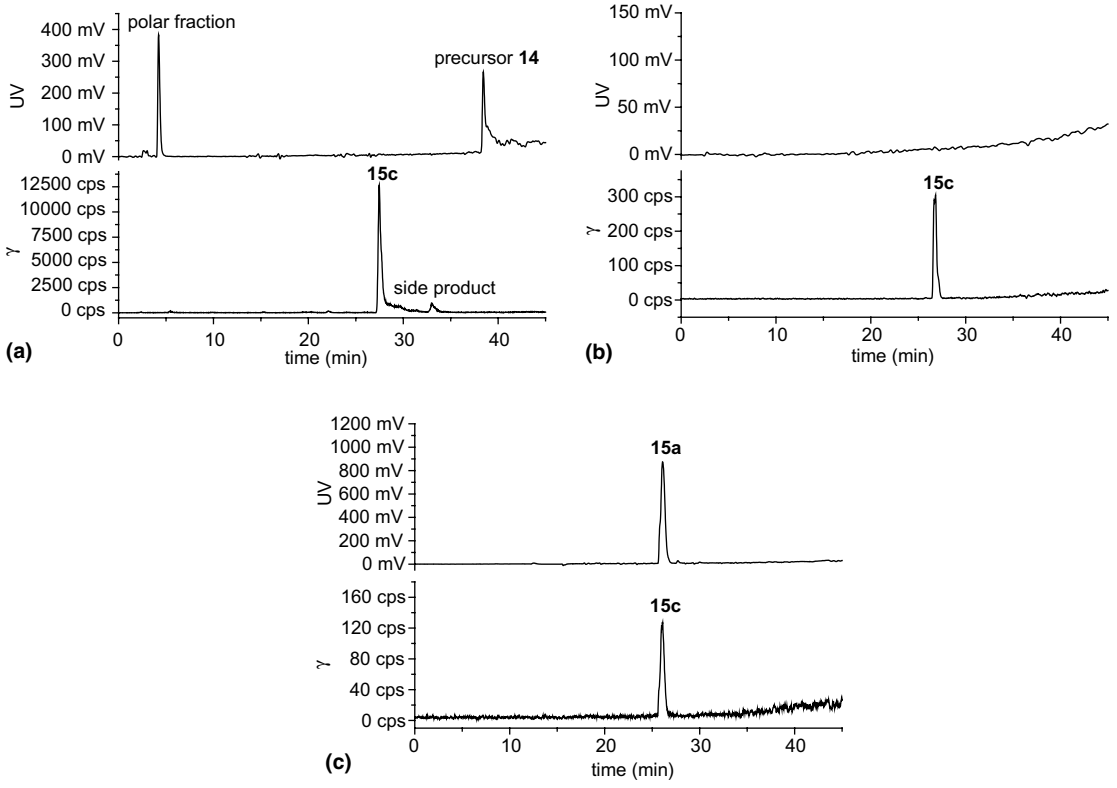


Figure 1. Representative HPLC chromatogram for (a) a reaction mixture of **15c**, (b) a quality control of **15c** and (c) a reference control of **15c** with 25 μg of its nonradioactive counterpart **15a**.

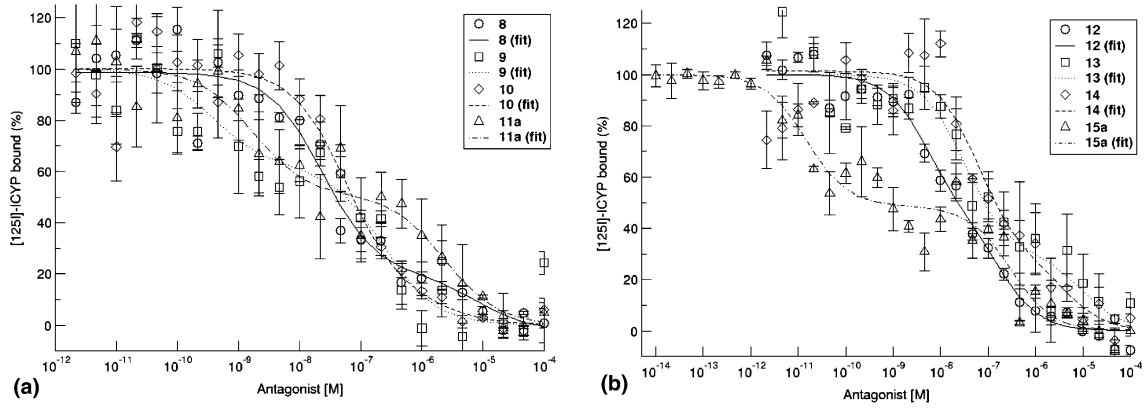


Figure 2. Competition of (a) racemic ICI 89,406 derivatives (**8**–**10**, **11a**). (b) (*S*)-Configurated ICI 89,406 derivatives (**12**–**14**, **15a**) with [^{125}I]ICYP binding to mouse ventricular membranes. Ordinate: specific [^{125}I]ICYP binding expressed as percentage of maximum binding in each experiment. Abscissa: concentration of ligands competing for specific binding of [^{125}I]ICYP. Each point represents the mean ± SEM ($n = 4$). See Scheme 4a and b for details of substituents.

(ACD/LogD Suite) for compounds **8**–**11a** and **12**–**15a** are listed in Table 1 to imply the changes of the lipophilicities caused by the chemical modifications of the lead compound ICI 89,406.

The esterified racemic silane **8** possesses significantly higher K_1- and K_2-values than its (*S*)-enantiomer **12**. While the K_1-value of **12** is reduced about 4-fold ($K_1 = 4.48$ nM vs $K_1 = 1.05$ nM) the K_2-value of **12** decreases from 1340 to 190 nM. This unequal reduction leads to a higher β_1-selectivity of **8** compared to compound **12** (299 vs 180). The same effect can be observed by changing the bulky TMS-group into an iodine. The resulting racemic β-AR ligand **9** is nearly threefold more β_1-selective than the (*S*)-compound **13** (1140 vs 430). Furthermore, both the racemic **9** and the corresponding (*S*)-ligand **13** possess higher β_1-selectivities than their TMS-counterparts **8** and **12**. In contrast to the pair **8** and **12**, the (*S*)-configurated ligand **13** ($K_1 = 15.7$ nM, $K_2 = 6700$ nM) exhibits a lower β_1- and β_2-affinity

Table 1. Inhibition constants and calculated β_1-AR selectivities of the ligands determined by a radioligand binding assay using mouse ventricular membrane preparations, plus calculated ligand log *P* and log *D* values.

Cpd.	K_1 (nM)[a]	K_2 (nM)[a]	β_1-Selectivity[b]	log *P*/log *D*[c]
8	4.48 ± 2.29	1340 ± 700	299 ± 53	5.10/4.10
12	1.05 ± 0.40	190 ± 16	180 ± 68	5.10/4.10
9	0.15 ± 0.01	171 ± 14	1140 ± 210	3.59/2.64
13	15.7 ± 9.0	6700 ± 3400	430 ± 140	3.59/2.64
10	14.0 ± 4.7	1000 ± 340	71 ± 2	4.80/2.28
14	14.4 ± 5.0	1090 ± 300	76 ± 12	4.80/2.28
11a	0.60 ± 0.24	500 ± 170	843 ± 268	3.29/0.79
15a	0.0049 ± 0.0008	140 ± 30	29700 ± 11500	3.29/0.79
ICI 89,406[d]	1.30 ± 0.20	160 ± 40	121 ± 14	1.57/0.21
2-I-ICI-H[d]	0.045 ± 0.005	12 ± 2	266 ± 28	3.18/1.80

[a] Displacement of specifically bound nonselective β-AR ligand [^{125}I]ICYP binding at β_1- and β_2-ARs expressed in mouse ventricular membrane preparations as mean ± SEM, $n = 4$.

[b] The ratios of the low- over the high-affinity inhibition constants (K_2/K_1) indicate the β_1-selectivities of the nonradioactive β_1-AR ligands, noted as mean ± SEM, $n = 4$.

[c] Log *P* values of the neutral form and log *D* values calculated by ACD/LogD Suite (log *D* = log *P* at physiological pH 7.4 with consideration of charged species).

[d] From Ref. 33.

than the racemic mixture **9**. ($K_1 = 0.15$ nM, $K_2 = 171$ nM).

The TMS-acids **10** ($K_1 = 14.0$ nM, $K_2 = 1000$ nM) and **14** ($K_1 = 14.4$ nM, $K_2 = 1090$ nM) have comparable β_1- and β_2-affinities, resulting in similar β_1-selectivities (71 vs 76). The replacement of a methoxycarbonyl group in **8** and **12** with a more polar carboxy moiety in **10** and **14** is associated with a decrease in β_1-affinity and selectivity. The β_1-affinity and selectivity of the racemic mixture **10** is reduced threefold in comparison to **8** and when the (*S*)-configurated **14** is compared with the methoxycarbonyl enantiomer **12** a nearly 14-fold decrease of β_1-affinity and a twofold decrease of β_1-selectivity can be observed ($K_1 = 14.4$ nM, β_1-selectivity 76 vs $K_1 = 1.05$ nM, β_1-selectivity 180). The β_1-affinity of the nonradioactive racemic target compound **11a** is reduced 4-fold in comparison to the esterified derivative **9** ($K_1 = 0.60$ nM vs $K_1 = 0.15$ nM), similar to the TMS-compounds **8** and **10**. The β_2-affinities of these derivatives differ in the same range resulting in comparable β_1-selectivities (**11a** 843 vs **9** 1140). The (*S*)-target compound **15a** shows a different behaviour. It has about 3000-fold more β_1-affinity than the (*S*)-ester **13** ($K_1 = 0.0049$ nM vs $K_1 = 15.7$ nM) while the β_2-affinity is reduced only 50-fold resulting in a huge β_1-selectivity (29700). In summary, the pair of acid derivatives **11a** and **15a** possesses completely different properties compared to the methyl esters **9** and **13**. The acid pair **11a** and **15a** is characterized by a very high β_1-affinity and selectivity of the (*S*)-enantiomer **15a**. In contrast the racemic mixture **9** of the ester pair **9** and **13** has the higher β_1-affinity and selectivity.

Compared to 2-I-ICI-H the substitution of the second phenyl core with a methyl ester or carboxylic acid moiety leads to a decrease of β_1-affinity but an increase of β_1-selectivity (compare Table 1 data of 2-I-ICI-H, **9** and **11a**). Furthermore, the two racemic iodine derivatives **9** and **11a** are more potent β_1-AR ligands than the lead compound ICI 89,406 caused by increased β_1-affinity and β_1-selectivity (compare Table 1 data of ICI 89,406, **9** and **11a**).

3.2. In vivo studies

The clearance of radioactivity from plasma after intravenous injection of **11b** or radioiodinated **15b** or **15c** into rats was rapid (Fig. 3). Predosing rats with nonradioactive **11a** had little effect (data not shown). The distribution of radioactivity in rat tissues at 20 min after injection of racemic **11b** given alone or 5 min after established β-AR antagonists is shown in Figure 4. Uptake of radioactivity in the myocardium and lung was low but predosing with the nonselective β-AR antagonist propranolol, nonradioactive **11a** and both doses (1 μg/kg or 100 nmol/kg) of the β_1-AR antagonist, ICI 89,406 reduced uptake. In contrast, predosing with the selective β_2-AR antagonist, ICI 118551, had little effect. None of the antagonists reduced the radioactivity in the liver or kidney (Fig. 4), or fat or muscle (data not

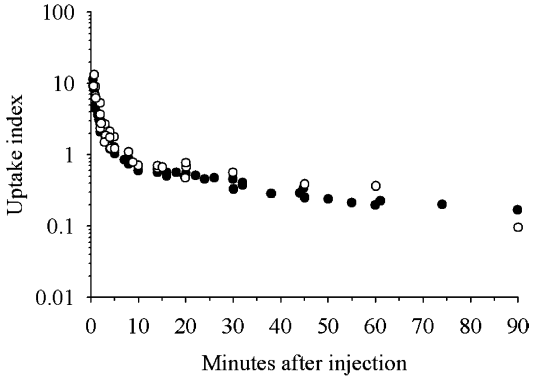


Figure 3. Clearance of radioactivity from plasma after *i.v.* injection of radioiodinated **11b** (●, 8 rats) or **15b**, **15c** (○, 11 rats). Up to six blood samples were taken from each rat.

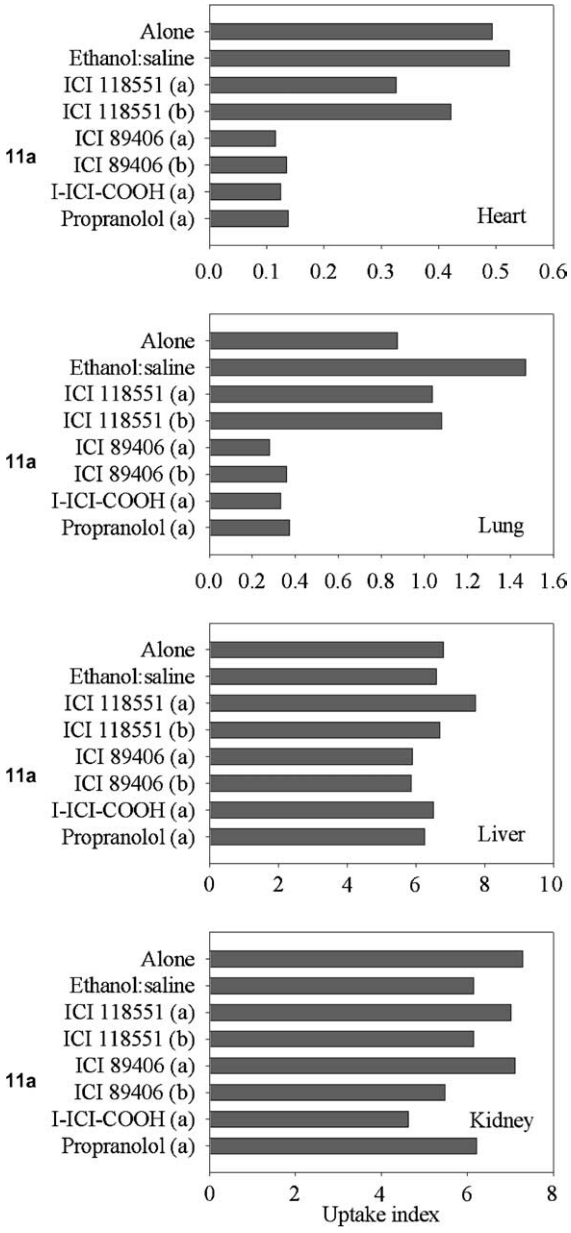


Figure 4. Radioactivity in heart, lung, liver or kidney at 20 min after *i.v.* injection of **11b** given alone or 5 min after *i.v.* injection of ethanol/saline, nonradioactive ICI 118551, nonradioactive ICI 89,406, nonradioactive **11a** (I-ICI-COOH) or propranolol ($a = 100\,\text{nmol mol kg}^{-1}$, $b = 1\,\mu\text{mol mol kg}^{-1}$).

$$\text{Uptake index} = \frac{\text{Tissue radioactivity (cpm)/tissue wet weight (g)}}{\text{Radioactivity injected (cpm)/body weight (g)}}.$$

Each bar represents uptake in a single rat.

shown). Uptake in the brain was negligible (uptake index < 0.02).

Tissue radioactivity after the injection of racemic **11b** or (*S*)-configurated **15b** or **15c** is illustrated in Figure 5. Uptake after radioiodinated (*S*)-ligands **15b** or **15c** was greater than that after racemic **11b** in the thyroid, lung and heart. Predosing with unlabelled **11a** or **15a** decreased the uptake of radioactivity in heart, lung and thyroid but not in other examined tissues.

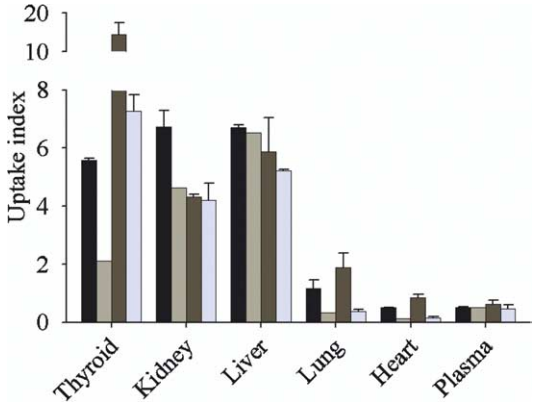


Figure 5. Radioactivity in tissues 20 min after *i.v.* injection of radioiodinated **11b** given alone (■) or 5 min after *i.v.* injection of unlabelled **11a** at $1\,\mu\text{mol kg}^{-1}$ (▭) or radioiodinated **15b**, **15c** given alone (■) or 5 min after *i.v.* injection of nonradioactive **15a** at $1\,\mu\text{mol kg}^{-1}$ (▭). Averages for two rats are shown. Bars indicate ranges.

Figure 6 shows the radioactivity in heart, liver, kidney and thyroid as a function of time after injection of radioiodinated **11b**, **15b** or **15c**. In the tissues, the maximum uptake was observed at the first sampling time, 2 min after injection and there was then a loss in radioactivity from heart, liver and kidney. In the heart, but not in the other tissues, uptake after radioiodinated **15b** or **15c** ((*S*)-configuration) was greater than that after **11b** (racemate). Radioacitivity in the thyroid, however, increased with time after injection. Predosing with nonradioactive **15a** decreased uptake in the heart but not in the other tissues.

Figure 7 shows the HPLC profiles of rat plasma at 2 min after injection of **15c**. The HPLC chromatogram after 25 min after injection had the same profile (data not shown). No radioactivity was detected at the reference retention time for this compound for samples obtained at either time after radioligand administration. The radioactivity in the plasma corresponded almost quantitatively to free iodide eluting in the polar fraction. A similar profile was obtained when **15c** was added to a sample of blood in vitro but when it was added to a sample of plasma parent **15c** was detected.

4. Discussion

4.1. Chemistry

Because of the clear clinical research need for a selective β_1-AR radioligand, the high affinity selective β_1-AR ligand, ICI 89,406 (75–195 fold β_1-selectivity),[33–37] was chosen as the lead compound for the development of radioligands with a 3-aryloxy-2-propanolamine subunit.

In comparison to the natural catecholamine agonists, this typical β-AR antagonist is characterized by an increased hydrophobicity of the aromatic ring system

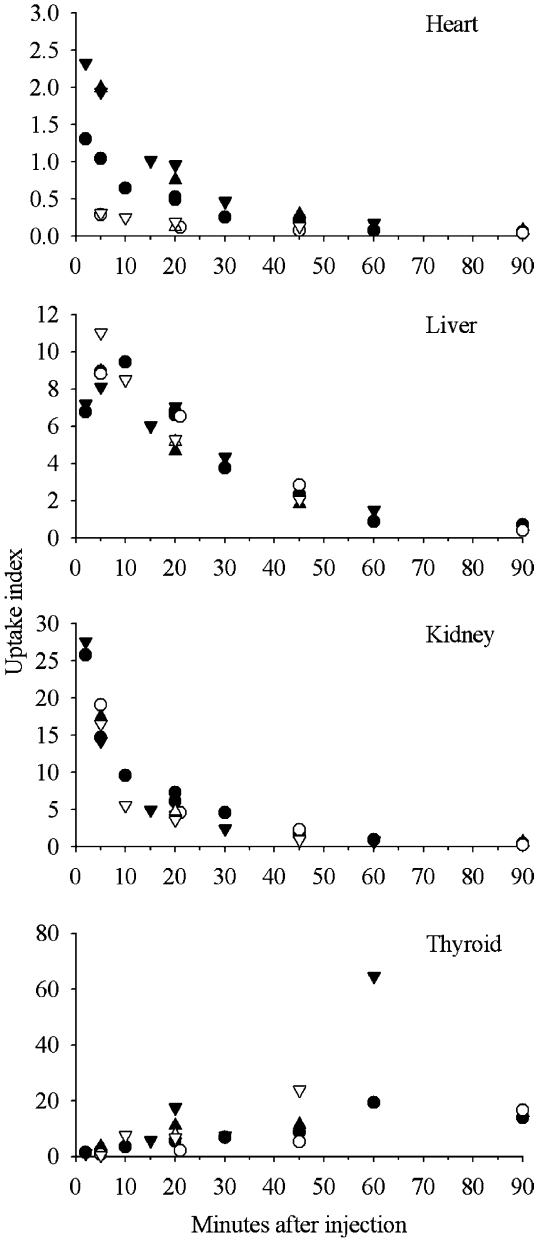


Figure 6. Uptake of radioactivity in heart, liver, kidney and thyroid after *i.v.* injection of radioiodinated target-compound into rats. Each symbol is the datum point for an individual rat. Radioiodinated **11b** was given alone (●, 9 rats) or 5 min after *i.v.* injection of nonradioactive **11a** at 1 μmol kg^{-1} (○, 4 rats). Radioiodinated **15b**, **15c** was given alone (▲, 10 rats) or 5 min after unlabelled **15a** at 1 μmol kg^{-1} (△, 5 rats). Note the differences in the scales for uptake indices.

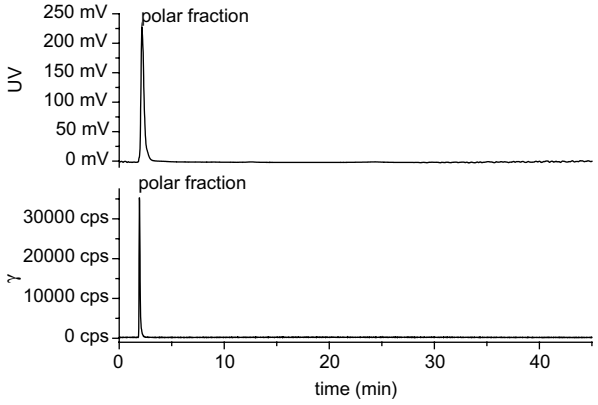


Figure 7. HPLC analysis of plasma taken from rats at 2 min after injection of **15c**.

substituted with a cyano moiety and by an increased distance between the amino group and this aromatic subunit, which is realized by a substitution of a phenoxymethylene moiety against the phenyl ring of the agonist.[43]

In previous work, we synthesized several racemic derivatives of ICI 89,406, mostly modified in the pattern of aromatic substituents, and examined them in in vitro binding studies. Nine compounds that possess an improved β_1-AR selectivity and affinity compared with ICI 89,406 were identified. Seven of them serve as nonradioactive counterparts or precursor compounds of candidate PET- and SPECT-radioligands. The remaining two compounds are useful to evaluate the (SAR) between the potential ligands and β_1-ARs.[33] We selected the potent racemic ligand, 2-I-ICI-H, that showed a higher β_1-selectivity and affinity (266, 0.045 nM) than ICI 89,406 (121, 1.3 nM) in the in vitro competition studies, for further evaluation and synthesized its radioiodinated counterpart 2-[^{125}I]I-ICI-H (Scheme 1). Due to its high degree of nonspecific binding in vitro and almost no specific binding to cardiac β_1-ARs in vivo this radiolabelled compound was not suitable as a radioligand for studies in vivo.[34]

The present work continues from these results. To reduce the lipophilicity and to avoid a high degree of nonspecific binding at blood plasma proteins, a polar moiety in 2-I-ICI-H was introduced (i.e. a carboxylate moiety). The resulting compounds **11a** (racemic) and **15a** ((*S*)-configuration) exhibited an amino-acid-like structure feature with an amino and a carboxylate group. Its predominant species at physiological pH (pH: 7.4) possess a polar zwitterionic structure similar to amino acids (95.6%, calculated by ACD/LogD Suite). The log *D* value of **11a** and **15a** (0.79) is significantly less than that for the iodo compound 2-I-ICI-H (1.80).

Usually the (*S*)-enantiomers of β-blockers show approximately a 100-fold higher affinity to β-ARs than the corresponding (*R*)-enantiomers.[44,45] Therefore, to increase the β_1-AR selectivity and affinity, we synthesized the (*S*)-enantiomers **15a**–**c** as well as the racemic iodinated compounds **11a**–**c**. The synthesized racemic and (*S*)-aryloxypropanolamines **8**–**11a** and **12**–**15a** were tested in binding studies in vitro to gain further information about the SAR of these derivatives.

β_1-ARs are a member of the rhodopsin/β_2 adrenergic receptor-like receptors, that represent a subfamily of the GPCRs (G protein-coupled receptors).[46] The binding sites of β-AR antagonists enclose the carboxylated side chain of Asp113 in transmembrane helix 3 (TM 3) of

β-ARs, that form a salt bridge between the positively charged amine group of the antagonists and the receptor.[47–49] This cationic centre is represented by the protonated secondary amine moiety of compounds **8**–**15a**. The phenoxyoxygen atom of the synthesized target compounds **8**–**15a** may be involved in a hydrogen bond with Asp312 in TM 7, that has been identified as a critical interaction point,[46,50] and the aryloxycore of the ligands **8**–**15a** may be stabilized by π-stacking with Phe289 and Phe290 of TM 6 similar to the proposed interactions of the agonist catechol ring and these aromatic amino acid residues.[48,51,52] In general the antagonist binding site of β-ARs is a nonsolvent exposed pocket, that is buried in the transmembrane core and differs from the binding site of the neurotransmitter agonists.[53–55]

The β_1-AR ligand pair **8** and **12** is characterized by stereospecific binding properties, that are known for the classical aryloxyalkylamine antagonists. The silylated (*S*)-compound **12** possesses a higher β_1-affinity than the racemic mixture **8** ($K_1 = 1.05$ nM vs $K_1 = 4.48$ nM). However, on changing the bulky TMS group into a iodo atom the affinity behaviour of the resulting β_1-AR ligands **9** and **13** is reversed. The enantiomeric pure (*S*)-compound **13** ($K_1 = 15.7$ nM) is about 100-fold less potent than the racemate **9** ($K_1 = 0.15$ nM). This unexpected fact may be based on a different binding orientation of **12** and **13** caused by the steric demand of the bulky TMS group on the one hand and a negatively polarized halogen atom on the other.

The TMS acid pair **10** and **14** looses β_1-affinity and selectivity compared to their esterified counterparts **8** and **12** (affinity: 3–13-fold decrease, selectivity: 2–4-fold decrease). Probably the negatively charged carboxylate moiety in combination with the TMS residue decreases the potency of the β_1-AR ligands **10** and **14** in comparison to **8** and **12** due to more repulsion forces between receptor and ligand. Both acid derivatives **10** and **14** possess similar β_1-affinities and selectivities (Table 1).

The target pair **11a** and **15a** shows different binding features. The iodinated (*S*)-acid **15a** is a selective high affinity β_1-AR ligand. This compound shows a 122-fold increased β_1-affinity ($K_1 = 0.0049$ nM vs $K_1 = 0.60$ nM) and a 35-fold higher selectivity (29700 vs 843) than the racemic mixture **11a**. In contrast to the TMS acids **10** and **14**, that loose β_1-affinity and selectivity in comparison to their TMS esters **8** and **12**, the iodinated acids **11a** and **15a** possess partially increased β_1-affinities and selectivities compared to their ester counterparts **9** and **13**. The stereospecific binding properties of **11a** and **15a** are similar to those of the known aryloxyalkylamine antagonists for which the (*S*)-enantiomers have a higher affinity than their corresponding racemates. It appears that the substitution of the methyl ester group with a polar carboxylate group leads to a stereospecific binding orientation of ligand and β_1-AR that preferentially binds the (*S*)-enantiomer. In summary, the in vitro binding studies identified the acids **11a** and especially **15a** as promising and potent β_1-AR ligands. Both were resynthesized radiochemically to evaluate the resulting radioligands **11b**–**c** and **15b**–**c** in studies in vivo.

4.2. Biodistribution studies

The first biodistribution study of **11b** in rats showed that at 20 min after intravenous injection of the radioligand, uptake of radioactivity in heart and lung was low compared with that in liver and kidney (Fig. 4). Predosing the rats with the nonselective β-AR antagonist propranolol, unlabelled **11a** and two doses (1 μg/kg or 100 nmol/kg) of the β_1-AR antagonist, ICI 89,406, reduced uptake. In contrast, predosing with the selective β_2-AR antagonist, ICI 118551, had little effect. None of the antagonists reduced the radioactivity in the liver or kidney (Fig. 4), or fat or muscle (data not shown). This result suggests that **11b** binds selectively to β_1-ARs in the heart and lung and that nonspecific binding is low in these tissues.

Uptake of radioactivity after injection of the radioiodinated (*S*)-enantiomer **15b** or **15c** was greater than that of the racemic compound **11b** in the heart, lung and thyroid but not in the other studied tissues (Fig. 5). Predosing with nonradioactive **11a** or **15b** reduced the tissue radioactivity in the heart and lung to similar values. This is consistent with published results that demonstrate that the (*S*)-enantiomers of β-blockers show higher affinity to β-AR than the corresponding racemates or (*R*)-enantiomers. In contrast although predosing did reduce the radioactivity in the thyroid, uptake after the (*S*)-enantiomer with predosing was greater than that after the racemate without predosing (Fig. 5).

Although the biodistribution at 20 min suggests that radioiodinated target-compounds bind selectively to β_1-AR in the heart and lung, the time–activity curves indicate that radioactivity was lost from all studied tissues with the exception of the thyroid (Fig. 6). In the heart (Fig. 6) clearance of the (*S*)-enatiomers **15b**–**c** was greater than that after the racemic compound **11b** and predosing with nonradioactive **11a** or **15a** reduced the maximal uptake but the time profile was not optimal for functional imaging. The data for the thyroid showed considerable scatter but there was a gradual increase in radioactivity following injection of both **11b** and **15b**–**c** with or without predosing. This increase in thyroid radioactivity suggested that free iodide ion was present, presumably due to metabolism of the radioligand. The HPLC analysis of plasma taken from rats at 2 and 25 min after injection of **15c** did not detect parent **15c** (Fig. 7). Radioactivity in the plasma eluted at the same time as free iodide ion. Rapid breakdown occurred when **15c** was added to rat blood in vitro although when it was added to plasma parent **15c** was detected. Therefore radioiodinated **11c** and **15c** appear to be unpromising radioligands for assessing β_1-AR in vivo in the rat. The rat, however, may metabolize β-AR-ligands more rapidly than other species. For example, comparative studies of metabolism of (*S*)-[^{11}C]CGP 12177 in rat, dog and man demonstrated significant metabolism in rat but

no detectable metabolism during the duration of a PET scan in dog and man.[56,57] Therefore the target compounds **11c** and **15c** should be evaluated in different animal models.

5. Conclusion

The newly synthesized nonradioactive compounds **11a** (racemate) and especially **15a** ((*S*)-enantiomer) possess excellent β_1-affinities and β_1-selectivities. In contrast to their esterified precursors **9** and **13**, where the racemate **9** has more β_1-AR affinity and selectivity than the (*S*)-isomer **13**, the pair **11a** and **15a** showed stereoselective binding behaviour to β_1-AR, that is characteristic for β_1-AR antagonists with such a 3-aryloxy-2-propanolamine structure. The (*S*)-enantiomer **15a** featured higher β_1-AR affinity (122-fold) and β_1-AR selectivity (35-fold) than **11a**. The radiochemically synthesized counterparts **11b–c** (racemic) and **15b–c** ((*S*)-enantiomer) were evaluated in vivo in the rat to test their suitability as SPECT-radioligands. Although the results argue against the suitability of **11c** and **15c** as promising SPECT-radioligands for assessing β_1-ARs in vivo in the rat because of rapid radioligand metabolism, the rat seems to be an inappropriate animal model. β-AR-ligands may be metabolized more rapidly in rats than in other species (mouse, dog, man) as demonstrated for (*S*)-[^{11}C]CGP 12177, which is structurally related to **11a–c** and **15a–c**. Therefore further studies in different animal models (e.g. nonhuman primate, mice) will be carried out with the aim to use these β_1-AR selective radioligands in the study of patients with cardiac diseases in the future.

6. Experimental section

6.1. Synthetic methods

All chemicals, reagents and solvents for the synthesis of the compounds were analytical grade and purchased from commercial sources.

The melting points (uncorrected) were determined on a Stuart Scientific SMP3 capillary melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker ARX 300 or AMX 400 spectrometer, respectively. Mass spectrometry was performed via a Varian MAT 212 (EI = 70 eV) spectrometer and a Bruker MALDI-TOF-MS Reflex IV (matrix: DHB). $[\alpha]_D$ values were determined on a Perkin–Elmer polarimeter 341. Elemental analysis was realized by a Vario EL III analyzer. Separation of the radiosynthesized compounds, analyses of the radiochemical yields and the radiochemical purity were performed by gradient radio-HPLC using a Knauer K-500 and a Latek P 402 pump, a Knauer K-2000 UV-detector (wavelength 254 nm) and a Crismatec Na(Tl) Scintibloc 51 SP51 γ-detector. Sample injection was carried out using a Rheodyne injector block (type 7125 incl. 200 μL loop). The recorded data were processed by the NINA radio-HPLC software (GE Functional Imaging GmbH).

6.2. Synthesis of the oxirane compounds 2a–d (Scheme 2)

6.2.1. 2-(2-Trimethylsilyl-phenoxymethyl)-oxirane 2a. 5.80 g (34.9 mmol) 2-(Trimethylsilyl)-phenol **1a**, prepared as previously described,[38,39] 19.11 g (139 mmol) bromomethyl-oxirane and 17.29 g (125 mmol) anhydrous K_2CO_3 were refluxed in 40 mL dry 2-butanone for 6 h under Ar atmosphere. The hot mixture was filtered and the filter cake was washed with 2-butanone. The combined filtrates were evaporated and the crude reaction product was purified by silica gel chromatography (hexane/diethyl ether 49:1) to give **2a** (R_f: 0.16) as a colourless liquid. Yield: 6.52 g (29.3 mmol), 84%. ^{1}H NMR (300 MHz, $CDCl_3$, TMS intern): δ [ppm]: 7.40–7.37 (m, 1H, H_{aryl}), 7.34–7.28 (m, 1H, H_{aryl}), 6.96 (dt, $^3J = 7.3$ Hz, $^4J = 1.0$ Hz, 1H, H_{aryl}), 6.79 (t, $^3J = 8.4$ Hz, 1H, H_{aryl}), 4.18 (dd, $^2J = 10.8$ Hz, $^3J = 3.3$ Hz, 1H, $1CH_2$), 3.97 (dd, $^2J = 11.1$ Hz, $^3J = 5.7$ Hz, 1H, $1CH_2$), 3.37–3.33 (m, 1H, CH), 2.89 (dd, $^2J = 5.0$ Hz, $^3J = 4.1$ Hz, 1H, $1CH_2$), 2.72 (dd, $^2J = 5.1$ Hz, $^3J = 2.7$ Hz, 1H, $1CH_2$), 0.29 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$, TMS intern): δ [ppm]: 163.96, 136.02, 131.57, 129.06, 121.83, 111.09, 69.53, 51.02, 45.56, −0.87. GC–MS (EI): m/z (intensity %): 222 ($M^{+\cdot}$, 13), 177 (100), 161 (43), 151 (45), 135 (31), 91 (46). Anal. Calcd for $C_{12}H_{18}O_2Si$: C, 64.82; H, 8.16. Found: C, 64.49; H, 8.31.

6.2.2. 2-(2-Iodo-phenoxymethyl)-oxirane 2b. 7.19 g (77.7 mmol) chloromethyl-oxirane, 4.80 g (21.8 mmol) 2-iodo-phenol **1b** and 15 mL 2 N NaOH were heated to 50 °C for 2.5 h. Then the mixture was extracted three times with CH_2Cl_2, the combined organic layers were dried ($MgSO_4$) and the solvent and volatile compounds were evaporated. The residue was distilled in vacuo fractionally to provide **2b** as a colourless liquid. Yield: 4.50 g (16.3 mmol), 75% (lit.[58]: 44%). bp (3.0 mbar): 140 °C (lit.[58]: 118–123 °C (0.2 Torr)). ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm]: 7.84 (dd, $^3J = 7.3$ Hz, $^4J = 1.6$ Hz, 1H, H_{aryl}), 7.38–7.34 (m, 1H, H_{aryl}), 6.91 (dd, $^3J = 8.4$ Hz, $^4J = 1.2$ Hz, 1H, H_{aryl}), 6.80 (dt, $^3J = 7.6$ Hz, $^4J = 1.5$ Hz, 1H, H_{aryl}), 4.35 (dd, $^2J = 11.2$ Hz, $^3J = 2.8$ Hz, 1H, $1CH_2$), 4.13 (dd, $^2J = 11.2$ Hz, $^3J = 5.2$ Hz, 1H, $1CH_2$), 3.48–3.44 (m, 1H, CH), 3.00–2.89 (m, 2H, $2CH_2$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm]: 157.55, 139.98, 129.91, 123.58, 113.22, 87.14, 70.12, 50.47, 45.13.

6.2.3. (*S*)-2-(2-Trimethylsilyl-phenoxymethyl)-oxirane 2c. Compound **2c** was prepared from 1.78 g (10.7 mmol) 2-(trimethylsilyl)-phenol **1a**, 5.55 g (21.5 mmol) (*S*)-glycidyl-3-nitrobenzene sulfonate and 5.28 g (38.2 mmol) anhydrous K_2CO_3 in 20 mL dry 2-butanone similar to the procedure described for **2a**. Yield: 1.82 g (8.2 mmol), 76%. ^{1}H NMR (300 MHz, $CDCl_3$, TMS intern): δ [ppm]: 7.39 (dd, $^3J = 7.2$ Hz, $^4J = 1.8$ Hz, 1H, H_{aryl}), 7.35–7.29 (m, 1H, H_{aryl}), 6.96 (dt, $^3J = 7.2$ Hz, $^4J = 0.6$ Hz, 1H, H_{aryl}), 6.79 (dd, $^3J = 8.1$ Hz, $^4J = 0.3$ Hz, 1H, H_{aryl}), 4.18 (dd, $^2J = 10.8$ Hz, $^3J = 3.6$ Hz, 1H, $1CH_2$), 3.97 (dd, $^2J = 10.8$ Hz, $^3J = 5.4$ Hz, 1H, $1CH_2$), 3.37–3.32 (m, 1H, CH), 2.90–

2.87 (m, 1H, $1CH_2$), 2.72 (dd, $^2J = 5.0$ Hz, $^3J = 2.6$ Hz, 1H, $1CH_2$), 0.30 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$, TMS intern): δ [ppm]: 163.04, 135.10, 130.65, 128.13, 120.91, 110.16, 68.63, 50.10, 44.63, −0.91. $[\alpha]_D^{20}$ +17.2 (*c* 1.0, C_2H_5OH). Anal. Calcd for $C_{12}H_{18}O_2Si$: C, 64.82; H, 8.16. Found: C, 64.43; H, 7.99.

6.2.4. (*S*)-2-(2-Iodo-phenoxymethyl)-oxirane 2d. To a solution of 5.00 g (22.7 mmol) 2-iodo-phenol **1b** in 568 mL dry toluene, 17.1 mL 1.3 M KOH (22.2 mmol) were added. After stirring for 30 min at RT, the mixture was evaporated to dryness. Acetonitrile was added and evaporated to a volume of approximately 190 mL. After heating to reflux, a solution of 5.88 g (22.7 mmol) (*S*)-glycidyl-3-nitrobenzene sulfonate in 170 mL dry acetonitrile was added dropwise. Thirty minutes after complete addition, the reaction mixture was cooled to RT and stirred for 2 h. The mixture was evaporated to dryness, 200 mL water and 200 mL CH_2Cl_2 were added and the organic layer was separated. The mixture was extracted two times with CH_2Cl_2, the combined organic layers were dried (Na_2SO_4) and the solvent and volatile compounds were evaporated. The residue was distilled in vacuo in a Kugelrohr and the high boiling fraction (bp (2.1 mbar): 150–210 °C) was purified by silica gel chromatography (PE/diethyl ether 9:1) to give **2d** (R_f: 0.14) as a colourless liquid. Yield: 5.69 g (20.6 mmol), 91%. ^1H NMR (300 MHz, $CDCl_3$, TMS intern): δ [ppm]: 7.76 (dd, $^3J = 7.5$ Hz, $^4J = 1.5$ Hz, 1H, H_{aryl}), 7.30–7.24 (m, 1H, H_{aryl}), 6.83 (dd, $^3J = 8.3$ Hz, $^4J = 1.4$ Hz, 1H, H_{aryl}), 6.74–6.69 (m, 1H, H_{aryl}), 4.26 (dd, $^2J = 11.4$ Hz, $^3J = 3.0$ Hz, 1H, $1CH_2$), 4.04 (dd, $^2J = 11.1$ Hz, $^3J = 5.1$ Hz, 1H, $1CH_2$), 3.40–3.35 (m, 1H, CH), 2.90–2.87 (m, 2H, 2 CH_2). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm]: 157.05, 139.51, 129.44, 123.10, 112.70, 86.66, 69.43, 50.04, 44.67. $[\alpha]_D^{20}$ +6.9 (*c* 1.0, C_2H_5OH). Anal. Calcd for $C_9H_9IO_2$: C, 39.16; H, 3.29. Found: C, 38.75; H, 3.33.

6.3. Synthesis of 2-aminoethyl-ureas 5-7 (Scheme 3)

6.3.1. *N*-[2-[(*tert*-Butoxycarbonyl)amino]ethyl]-*N*′-(4-methoxycarbonyl-phenyl)-urea 5. 2.14 g (13.4 mmol) *N*-Mono(*tert*-butyloxycarbonyl)ethylenediamine **3** were dissolved in 9 mL dry ether. At 0 °C 2.37 g (13.4 mmol) 4-methoxycarbonyl-phenylisocyanate **4**, prepared using a procedure similar to that published elsewhere,[59] in 15 mL dry ether were added within 30 min. The mixture was left at +4 °C for 13 h and filtered. The filter cake was washed with 50 mL ether and dried in vacuo to provide **5** as a colourless solid. Yield: 3.95 g (11.7 mmol), 88%. mp: 172 °C. ^1H NMR (300 MHz, DMSO-d_6): δ [ppm]: 8.96 (s, 1H, NH), 7.82 (d, $^3J = 8.4$ Hz, 2H, H_{aryl}), 7.50 (d, $^3J = 8.4$ Hz, 2H, H_{aryl}), 6.79 (broad, s, 1H, NH), 6.31 (t, $^3J = 4.8$ Hz, 1H, NH), 3.79 (s, 3H, CH3), 3.14 (q, $^3J = 6.0$ Hz, 2H, CH_2), 3.01 (t, $^3J = 5.8$ Hz, 2H, CH_2), 1.36 (s, 9H, $3CH_3$). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ [ppm]: 187.63, 166.14, 154.95, 145.30, 130.42, 121.81, 116.84, 77.79, 51.75, 40.41, 39.27, 28.35. Anal. Calcd for $C_{16}H_{23}N_3O_5$: C, 56.96; H, 6.87; N, 12.46. Found: C, 56.58; H, 6.80; N, 12.54.

6.3.2. *N*-(2-Amino-ethyl)-*N*′-(4-methoxycarbonyl-phenyl)-urea hydrochloride 6. 3.37 g (10.0 mmol) *N*-[2-[(*tert*-Butoxycarbonyl)amino]ethyl]-*N*′-(4-methoxycarbonyl-phenyl)-urea **5** were dissolved in 7 mL of a conc. HCl/MeOH mixture (1:1, v/v). The solvents were evaporated at 50 °C in vacuo (600 → 30 mbar) within 1.5 h. Then 30 mL dry acetone were added and the solvent was removed in vacuo. This procedure was repeated two times. Thereafter the product was suspended in 25 mL acetone, stirred for 30 min, filtered and dried in vacuo to provide **6** as a colourless solid. Yield: 2.61 g (9.5 mmol), 95%. mp: 215 °C, decomposition. ^1H NMR (300 MHz, DMSO-d_6, TMS intern): δ [ppm]: 9.68 (s, 1H, NH), 8.05 (broad, s, 3H, NH_3^+), 7.83–7.80 (m, 2H, H_{aryl}), 7.56–7.52 (m, 2H, H_{aryl}), 6.93 (t, $^3J = 6.0$ Hz, 1H, NH), 3.77 (s, 3H, CH_3), 3.33 (q, $^3J = 6.1$ Hz, 2H, CH_2), 2.88 (broad, s, 2H, CH_2). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ [ppm]: 166.13, 155.54, 145.26, 130.42, 121.91, 116.93, 51.82, 51.77, 37.26. Anal. Calcd for $C_{11}H_{16}ClN_3O_3$: C, 48.27; H, 5.89; N, 15.35. Found: C, 48.08; H, 5.91; N, 15.28.

6.3.3. *N*-(2-Amino-ethyl)-*N*′-(4-methoxycarbonyl-phenyl)-urea 7. 1.20 g (4.38 mmol) *N*-(2-Amino-ethyl)-*N*′-(4-methoxycarbonyl-phenyl)-urea hydrochloride **6** were dissolved in 24 mL water and 438 μL 10 N NaOH. The aqueous solution was refluxed with 50 mL $CHCl_3$ for 20 min and the layers were separated. This procedure was repeated four times. The combined organic layers were dried (Na_2SO_4) and the solvent was evaporated to give **7** as a colourless solid. Yield: 825 mg (3.48 mmol), 79%. mp: 190 °C, decomposition. ^1H NMR (300 MHz, DMSO-d_6): δ [ppm]: 8.97 (broad, s, 1H, NH), 7.81 (d, $^3J = 8.7$ Hz, 2H, H_{aryl}), 7.50 (d, $^3J = 8.7$ Hz, 2H, H_{aryl}), 6.35 (t, $^3J = 5.6$ Hz, 1H, NH), 3.78 (s, 3H, CH_3), 3.08 (q, $^3J = 5.9$ Hz, 2H, CH_2), 2.62 (t, $^3J = 6.2$ Hz, 2H, CH_2). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ [ppm]: 166.13, 155.01, 145.44, 130.42, 121.67, 116.78, 51.72, 42.56, 41.73. Anal. Calcd for $C_{11}H_{15}N_3O_3$: C, 55.69; H, 6.37; N, 17.71. Found: C, 55.88; H, 6.32; N, 17.46.

6.4. Synthesis of the 3-aryloxy-2-propanolamine derivatives 8–15 (Scheme 4a and b)

6.4.1. (±)-*N*-(4-Methoxycarbonyl-phenyl)-*N*′-[2-[3-(2-trimethylsilyl-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea 8 and its (*S*)-enantiomer 12. *N*-(2-Amino-ethyl)-*N*′-(4-methoxycarbonyl-phenyl)-urea **7** was dissolved in hot anhydrous acetonitrile (2.6 mL/mmol) under Ar atmosphere. An equimolar amount of 2-(2-trimethylsilyl-phenoxymethyl)-oxirane **2a** or its (*S*)-enantiomer **2c** was added, and the mixture was stirred under reflux for 2.5 h, at RT for 12 h, and again under reflux for 7.5 h. The reaction mixture was cooled to RT and filtered, the filter cake was washed with acetonitrile and the combined evaporated filtrates were purified by silica gel chromatography (ethyl acetate/MeOH 9:1) to give **8** as a colourless hygroscopic oil that solidified after some

weeks at 4 °C, and its (*S*)-enantiomer **12** (R_f: 0.15) as a colourless hygroscopic solid, respectively.

6.4.2. (±)-*N*-(4-Methoxycarbonyl-phenyl)-*N*′-[2-[3-(2-trimethylsilyl-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea 8. Yield: 55%. mp: 50–52 °C. ^{1}H NMR (300 MHz, $CDCl_3$, TMS intern): δ [ppm]: 8.25 (broad, s, 1H, NH), 7.87 (d, $^3J = 8.7$ Hz, 2H, H_{aryl}), 7.41–7.25 (m, 4H, H_{aryl}), 6.95 (dt, $^3J = 7.3$ Hz, $^4J = 0.7$ Hz, 1H, H_{aryl}), 6.75 (d, $^3J = 8.4$ Hz, 1H, H_{aryl}), 6.08 (t, $^3J = 5.3$ Hz, 1H, NH), 4.16–3.87 (m, 3H, CH_2CH), 3.84 (s, 3H, CH_3), 3.38–3.33 (m, 2H, CH_2), 3.08 (broad, s, 2H, OH and NH), 2.94–2.77 (m, 4H, $2CH_2$), 0.25 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$, TMS intern): δ [ppm]: 166.93, 162.98, 156.26, 143.85, 135.26, 130.88, 127.66, 123.86, 121.06, 118.08, 110.26, 77.19, 69.96, 68.64, 52.02, 49.63, 39.98, −0.60. MS (MALDI-TOF): m/z: 460 $(M+H)^+$, 482 $(M+Na)^+$. Anal. Calcd for $C_{23}H_{33}N_3O_5Si{\cdot}0.5H_2O$: C, 58.95; H, 7.53; N, 8.97. Found: C, 58.85; H, 7.16; N, 8.51.

6.4.3. (*S*)-*N*-(4-Methoxycarbonyl-phenyl)-*N*′-[2-[3-(2-trimethylsilyl-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea 12. Yield: 35%. mp: 75–76 °C. ^{1}H NMR (300 MHz, $CDCl_3$, TMS intern): δ [ppm]: 8.05 (broad, s, 1H, NH), 7.87 (d, $^3J = 9.0$ Hz, 2H, H_{aryl}), 7.39–7.25 (m, 4H, H_{aryl}), 6.94 (dt, $^3J = 7.3$ Hz, $^4J = 0.8$ Hz, 1H, H_{aryl}), 6.74 (d, $^3J = 8.4$ Hz, 1H, H_{aryl}), 5.48 (t, $^3J = 5.3$ Hz, 1H, NH), 4.12–3.90 (m, 3H, CH_2CH), 3.84 (s, 3H, CH_3), 3.33 (q, $^3J = 5.4$ Hz, 2H, CH_2), 2.89–2.73 (m, 4H, $2CH_2$), 2.44 (broad, s, 2H, OH and NH), 0.24 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$, TMS intern): δ [ppm]: 166.87, 162.96, 156.00, 143.77, 135.21, 130.86, 127.63, 123.86, 120.97, 118.05, 110.17, 77.14, 69.97, 68.87, 51.96, 49.57, 40.23, −0.65. MS (MALDI-TOF): m/z: 460 $(M+H)^+$, 482 $(M+Na)^+$. $[\alpha]_D^{20}$ −12.5 (*c* 1.0, C_2H_5OH). Anal. Calcd for $C_{23}H_{33}N_3O_5Si{\cdot}0.5H_2O$: C, 58.95; H, 7.53; N, 8.97. Found: C, 59.11; H, 7.23; N, 8.72.

6.4.4. (±)-*N*-[2-[3-(2-Iodo-phenoxy)-2-hydroxy-propylamino]-ethyl]-*N*′-(4-methoxycarbonyl-phenyl)-urea 9 and its (*S*)-enantiomer 13. 1.00 mmol *N*-(2-Amino-ethyl)-*N*′-(4-methoxycarbonyl-phenyl)-urea hydrochloride **6**, an equimolar amount of 2-(2-iodo-phenoxymethyl)-oxirane **2b** or its (*S*)-enantiomer **2d** and 1.05 equiv of 10 N NaOH were heated in approximately 3.0 mL *n*-propanol and 0.5 mL water up to 90 °C for 30–60 min. Water was added at RT until the mixture gets a slight turbidity. The suspension was cooled down to +4 °C for about 20 h, the product was filtered off, washed with water, after that with ethyl acetate, and finally dried in vacuo. The raw products were recrystallized two times from a ethyl acetate/acetonitrile mixture (8:1 and 10:1) to provide **9** and **13** as colourless solids, respectively.

6.4.5. (±)-*N*-[2-[3-(2-Iodo-phenoxy)-2-hydroxy-propylamino]-ethyl]-*N*′-(4-methoxycarbonyl-phenyl)-urea 9. Yield: 44%. mp. 137 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]: 9.00 (s, 1H, NH), 7.81 (d, $^3J = 8.4$ Hz, 2H, H_{aryl}), 7.74 (dd, $^3J = 7.7$ Hz, $^4J = 1.5$ Hz, 1H, H_{aryl}), 7.50 (d, $^3J = 8.7$ Hz, 2H, H_{aryl}), 7.34–7.28 (m, 1H, H_{aryl}), 6.99 (dd, $^3J = 8.4$ Hz, $^4J = 0.9$ Hz, 1H, H_{aryl}), 6.72 (dt, $^3J = 7.5$ Hz, $^4J = 1.2$ Hz, 1H, H_{aryl}), 6.34 (t, $^3J = 5.1$ Hz, 1H, NH), 4.85 (broad, s, 1H, OH), 4.00–3.90 (m, 3H, CH_2CH), 3.79 (s, 3H, CH_3), 3.19 (q, $^3J = 5.7$ Hz, 2H, CH_2), 2.85–2.64 (m, 4H, $2CH_2$). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ [ppm]: 166.09, 157.25, 154.94, 145.40, 139.02, 130.43, 129.82, 122.73, 121.69, 116.77, 112.91, 86.74, 71.62, 68.08, 52.39, 51.75, 49.21, 32.51. MS (MALDI-TOF): m/z: 514 $(M+H)^+$. Anal. Calcd for $C_{20}H_{24}IN_3O_5$: C, 46.80; H, 4.71; N, 8.19. Found: C, 46.48; H, 4.32; N, 7.93.

6.4.6. (*S*)-*N*-[2-[3-(2-Iodo-phenoxy)-2-hydroxy-propylamino]-ethyl]-*N*′-(4-methoxycarbonyl-phenyl)-urea 13. Yield: 37%. mp: 134–135 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]: 9.02 (s, 1H, NH), 7.81 (d, $^3J = 8.7$ Hz, 2H, H_{aryl}), 7.74 (dd, $^3J = 7.8$ Hz, $^4J = 1.5$ Hz, 1H, H_{aryl}), 7.50 (d, $^3J = 8.7$ Hz, 2H, H_{aryl}), 7.34–7.28 (m, 1H, H_{aryl}), 6.99 (dd, $^3J = 8.3$ Hz, $^4J = 1.4$ Hz, 1H, H_{aryl}), 6.72 (dt, $^3J = 7.4$ Hz, $^4J = 1.2$ Hz, 1H, H_{aryl}), 6.36 (t, $^3J = 5.4$ Hz, 1H, NH), 4.93 (broad, s, 1H, OH), 4.03–3.90 (m, 3H, CH_2CH), 3.78 (s, 3H, CH_3), 3.19 (q, $^3J = 5.9$ Hz, 2H, CH_2), 2.85–2.64 (m, 4H, $2CH_2$). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ [ppm]: 166.13, 157.24, 154.98, 145.42, 139.04, 130.44, 129.84, 122.76, 121.71, 116.78, 112.90, 86.74, 71.63, 68.08, 52.40, 51.77, 49.22, 32.95. $[\alpha]_D^{20}$ −12.3 (*c* 1.0, C_2H_5OH). Anal. Calcd for $C_{20}H_{24}IN_3O_5$: C, 46.80; H, 4.71; N, 8.19. Found: C, 46.78; H, 4.55; N, 8.39.

6.4.7. (±)-*N*-(4-Carboxy-phenyl)-*N*′-[2-[3-(2-trimethylsilyl-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea 10 and its (*S*)-enantiomer 14. 1 Equiv (±)-*N*-(4-methoxycarbonyl-phenyl)-*N*′-[2-[3-(2-trimethylsilyl-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea **8** or its (*S*)-enantiomer **12** and 11.1 equiv 1 N NaOH were stirred at RT for 66–93 h. The pH value was adjusted to 6–7 with 1 N HCl, the product was filtered off, washed with water and stirred under reflux in a ethyl acetate/acetonitrile mixture (1:1). The mixture was cooled down to −20–(−30) °C for several hours, the filtered compounds were washed with ethyl acetate/acetonitrile (1:1) and dried in vacuo to provide **10** and its (*S*)-enantiomer **14** as colourless solids, respectively.

6.4.8. (±)-*N*-(4-Carboxy-phenyl)-*N*′-[2-[3-(2-trimethylsilyl-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea 10. Yield: 77%. mp: 175–177 °C. ^{1}H NMR (300 MHz, $CDCl_3$, TMS intern): δ [ppm]: 9.39 (broad, s, 1H, NH), 7.80 (d, $^3J = 8.7$ Hz, 2H, H_{aryl}), 7.47 (d, $^3J = 8.4$ Hz, 2H, H_{aryl}), 7.29 (d, $^3J = 7.2$ Hz, 2H, H_{aryl}), 6.92–6.89 (m, 3H, NH, H_{aryl}), 5.29 (broad, s, 3H, NH, OH, COOH), 4.04–3.92 (m, 3H, CH_2CH), 3.27 (d, $^3J = 5.4$ Hz, 2H, CH_2), 2.93–2.74 (m, 4H, $2CH_2$), 0.24 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$, TMS intern): δ [ppm]: 168.56, 163.33, 155.39, 144.40, 134.72, 131.04, 130.41, 126.79, 125.07, 120.41, 116.57, 110.54, 70.22, 67.55, 52.02, 48.94, 38.46, −0.57. MS (MALDI-TOF): m/z: 446 $(M+H)^+$,

468 $(M+Na)^+$. Anal. Calcd for $C_{22}H_{31}N_3O_5Si$: C, 59.30; H, 7.01; N, 9.43. Found: C, 59.51; H, 6.65; N, 9.12.

6.4.9. (*S*)-*N*-(4-Carboxy-phenyl)-*N*′-[2-[3-(2-trimethylsilyl-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea 14. Yield: 69%. mp: 185–186 °C. ^{1}H NMR (300 MHz, $CDCl_3$, TMS intern): δ [ppm]: 9.42 (broad, s, 1H, NH), 7.80 (d, $^3J = 8.4$ Hz, 2H, H_{aryl}), 7.47 (d, $^3J = 8.7$ Hz, 2H, H_{aryl}), 7.29 (d, $^3J = 7.2$ Hz, 2H, H_{aryl}), 6.97–6.88 (m, 3H, NH, H_{aryl}), 6.11 (broad, s, 3H, NH, OH, COOH), 3.99–3.90 (m, 3H, CH_2CH), 3.25–2.69 (m, 6H, $3CH_2$), 0.24 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$, TMS intern): δ [ppm]: 168.55, 163.39, 155.39, 144.30, 134.40, 131.04, 130.37, 126.79, 125.53, 120.36, 116.50, 110.50, 70.29, 67.88, 52.28, 49.11, 38.81, −0.59. MS (MALDI-TOF): m/z: 446 $(M+H)^+$, 468 $(M+Na)^+$. $[\alpha]_D^{20}$ −13.5 (c 0.067, H_2O/C_2H_5OH 2/1). Anal. Calcd for $C_{22}H_{31}N_3O_5Si$: C, 59.30; H, 7.01; N, 9.43. Found: C, 59.38; H, 6.64; N, 9.09.

6.4.10. (±)-*N*-(4-Carboxy-phenyl)-*N*′-[2-[3-(2-iodo-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea 11a and its (*S*)-enantiomer 15a. 1 Equiv (±)-*N*-[2-[3-(2-iodo-phenoxy)-2-hydroxy-propylamino]-ethyl]-*N*′-(4-methoxycarbonyl-phenyl)-urea **9** or its (*S*)-enantiomer **13** and 11.1 equiv 1 N NaOH were stirred under reflux for 1–2 h and then at RT for 2–14 h. The pH value was adjusted to 6.2–6.8 with 1 N HCl, the product was filtered off and stirred under reflux in an ethyl acetate/methanol mixture (10:1). The mixture was cooled down to −20–(−30) °C for several hours, the filtered compounds were washed with ethyl acetate and dried in vacuo. The products were suspended in an ethyl acetate/methanol mixture (5:1), the mixture was heated under reflux for 2 h, cooled down to −30 °C for 17 h and filtered. The products were washed with methanol to provide **11a** and its (*S*)-enantiomer **15a** as colourless solids, respectively.

6.4.11. (±)-*N*-(4-Carboxy-phenyl)-*N*′-[2-[3-(2-iodo-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea 11a. Yield: 48%. mp: 185–186 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]: 9.17 (s, 1H, NH), 7.80 (d, $^3J = 8.7$ Hz, 2H, H_{aryl}), 7.74 (dd, $^3J = 7.8$ Hz, $^4J = 1.5$ Hz, 1H, H_{aryl}), 7.47 (d, $^3J = 8.7$ Hz, 2H, H_{aryl}), 7.35–7.29 (m, 1H, H_{aryl}), 7.00 (dd, $^3J = 8.1$ Hz, $^4J = 0.9$ Hz, 1H, H_{aryl}), 6.73 (dt, $^3J = 7.5$ Hz, $^4J = 1.1$ Hz, 1H, H_{aryl}), 6.69 (t, $^3J = 3.8$ Hz, 1H, NH), 6.37 (broad, s, 3H, NH, OH, COOH), 4.03–3.97 (m, 3H, CH_2CH), 3.24 (q, $^3J = 5.7$ Hz, 2H, CH_2), 2.93–2.73 (m, 4H, $2CH_2$). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ [ppm]: 168.00, 157.18, 155.20, 144.51, 139.01, 130.43, 129.80, 122.79, 116.59, 112.93, 86.73, 71.52, 67.54, 52.02, 49.03, 33.50. MS (MALDI-TOF): m/z: 500 $(M+H)^+$, 522 $(M+Na)^+$. Anal. Calcd for $C_{19}H_{22}IN_3O_5$: C, 45.71; H, 4.44; N, 8.42. Found: C, 45.52; H, 4.24; N, 8.20.

6.4.12. (*S*)-*N*-(4-Carboxy-phenyl)-*N*′-[2-[3-(2-iodo-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea 15a. Yield: 33%. mp: 193–194 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm]: 9.24 (s, 1H, NH), 7.80 (d, $^3J = 8.7$ Hz, 2H, H_{aryl}), 7.74 (dd, $^3J = 7.6$ Hz, $^4J = 1.5$ Hz, 1H, H_{aryl}), 7.46 (d, $^3J = 8.4$ Hz, 2H, H_{aryl}), 7.34–7.29 (m, 1H, H_{aryl}), 7.00 (d, $^3J = 7.5$ Hz 1H, H_{aryl}), 6.80 (broad, s, 1H, NH), 6.75–6.70 (m, 1H, H_{aryl}), 4.33 (broad, s, 3H, NH, OH, COOH), 4.03–3.97 (m, 3H, CH_2CH), 3.27–2.77 (m, 6H, $3CH_2$). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ [ppm]: 168.33, 157.19, 155.33, 144.40, 139.06, 130.45, 129.88, 122.87, 116.63, 112.97, 86.76, 71.49, 67.35, 51.90, 48.97, 33.04. MS (MALDI-TOF): m/z: 500 $(M+H)^+$. $[\alpha]_D^{20}$ −4.5 (c 0.067, H_2O/C_2H_5OH 2/1). Anal. Calcd for $C_{19}H_{22}IN_3O_5$: C, 45.71; H, 4.44; N, 8.42. Found: C, 45.47; H, 4.25; N, 8.29.

6.4.13. (±)-*N*-(4-Carboxy-phenyl)-*N*′-[2-[3-(2-[^{125}I]iodo-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea 11b, the [^{123}I]iodo-derivative 11c and its (*S*)-enantiomers 15b–c. A suspension of 3.3 mg (24.7 μmol) N-chlorosuccinimide (NCS) in 20 μL water for injection were added to a solution of 0.3 mg (0.67 μmol) (±)-*N*-(4-carboxy-phenyl)-*N*′-[2-[3-(2-trimethylsilyl-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea **10** or its (*S*)-enantiomer **14** in 12 μL acetic acid (HOAc) and 12 μL 8% $NaOAc{\cdot}3H_2O$ in HOAc. The radiosynthesis was started by adding this mixture to 6–49 MBq n.c.a. [^{125}I]NaI in 5–14 μL 0.05 N NaOH and 75–316 MBq n.c.a. [^{123}I]NaI in 5–31 μL 0.05 N NaOH, respectively. The reaction mixture was vortexed and allowed to stand 2 min at RT. The reaction was terminated by the addition of 100 μL eluent B (see below).

The diluted reaction mixture was injected onto a gradient RP-HPLC-chromatograph with a Nucleosil 100 column (C-18.5 μ250×4.6 mm) with a corresponding precolumn (20×4.6 mm) and combined γ-/UV-detectors to isolate the radiolabelled compounds **11b**, **11c**, **15b** and **15c**, respectively. Radiochemical yield for **11b** and **15b**: 78%. Radiochemical yield for **11c** and **15c**: 41%.

HPLC conditions:	Eluent A:	CH_3CN/H_2O/TFA 950/50/1
	Eluent B:	CH_3CN/H_2O/TFA 50/950/1
	Time program:	Eluent B from 92% to 50% within 45 min and then from 50% to 92% within 10 min
	Flow:	1.5 mL/min
	λ:	254 nm
	R_t (product fraction):	27.0–29.0 min (maximum 27.6 min, average).

6.5. Quality control

The product fraction (50 μL) was re-injected into the HPLC column. The quality control did not show any impurities within the γ-range. Only the injection peak was detectable within the UV range.

6.6. Reference control

The radioiodinated products **11b**, **11c**, **15b** or **15c** were verified by concentrating 25 μL of the isolated γ-fraction with 25 μg of the nonradioactive compounds **11a** and **15a**, each dissolved in 25 μL eluent B. The concentrated 50 μL mixtures were again injected into the HPLC column. Both the radiolabelled product and the nonradioactive reference standard corresponded to each other.

7. Biology

7.1. Tissue preparation

Microsomes were prepared by homogenizing ventricles from DBA mice at 4 °C for 90 s in 1 mL of a buffer A containing 10 mM EDTA, 10 mM HEPES, 0.1 mM benzamidine (pH 7.4), using a Polytron PT 3000 (Kinematica, Lucerne, Switzerland). Homogenates were centrifuged at $45{,}000 \times g_{max}$ for 15 min at 4 °C. The pellets were resuspended again in 1 mL of a buffer B containing 1 mM EDTA, 10 mM HEPES, 0.1 mM benzamidine (pH 7.4) and recentrifuged at $45{,}000 \times g_{max}$ for 15 min at 4 °C. The pellets were resuspended in 1 mL of buffer B and centrifuged at $10{,}000 \times g_{max}$ for 10 min at 4 °C. The supernatants were recentrifuged at $45{,}000 \times g_{max}$ for 15 min at 4 °C. The pellets, partially enriched membranes, were resuspended in buffer C (50 mM Tris·HCl, 5 mM $MgCl_2$ (pH 7.4)), and stored frozen at −80 °C.

For competition binding studies, the prepared membranes were resuspended in buffer D (10 mM Tris HCl, 154 mM NaCl, 0.1 mM ascorbic acid, pH 7.4). 15 μg of membranes were incubated with a constant concentration of [^{125}I]ICYP (80 pM) and with varying concentrations (1 pM–100 μM) of compounds **8**–**11a** and **12**–**15a** (Scheme 4). Reactions were conducted at 37 °C for 60 min. Reactions were stopped by filtering onto Whatman GF/B filters and washed with 0.9% NaCl. The membrane bound radioactivity was determined in a γ-scintillation counter. Competition binding curves were analyzed by nonlinear regression analysis as previously described.[60–62]

7.2. Animals

Adult male Sprague Dawley rats (250–320 g) were anaesthetized by isoflurane/N_2O/O_2 and catheters (o.d. 1 mM) were inserted into the ventral tail artery and one lateral tail vein of each rat. Animals were allowed to recover from the anaesthesia for 2–3 h and during the studies they were conscious but under light restraint. Each radioiodinated compound (**11b**, **15b** or **15c**) at 2–7 MBq kg^{-1} body weight) was injected as a bolus (1 μL g^{-1} body weight) via the tail vein. Aliquots of each injectate were diluted in ethanol/saline and measured to determine the radioactivity injected into each animal. In some animals, nonradioactive antagonists were injected as a bolus (1 μL g^{-1} body weight) via the tail vein 5 min before injection of the radioligand. Ligands used were **11a** or **15a** (1 μmol kg^{-1}), the selective β_1-AR antagonist ICI 89,406 (1 μmol kg^{-1} or 100 nmol kg^{-1}), the selective β_2-AR antagonist, ICI 118551 (1 μmol kg^{-1} or 100 nmol kg^{-1}) or the nonselective β-AR antagonist, propranolol (as HCl salt; 1 μmol kg^{-1}). Six sequential arterial blood samples (ca. 100 μL) were taken from each animal. An aliquot of whole blood was taken and the remainder centrifuged to separate the plasma. Animals were sacrificed by intravenous injection of sodium pentobarbitone (Euthatal) at 200 mg (kg body weight)$^{-1}$ at selected times after injection of radioligand and tissues were rapidly removed.

In some animals, heads were removed using large scissors, the brain removed from the cranium and brain regions dissected. In all animals, the thorax was opened by an incision on each side of the sternum. The heart and lungs were removed together. The heart was dissected into five regions, left and right atrial walls, left and right ventricular walls and interventricular septum, excluding the heart valves. The individual lobes of the lung were separated and samples (ca. 50 mg) taken from each lobe were combined. Samples (100–200 mg) from liver, kidney and striated muscle were also removed and a sample of urine taken from the bladder. Tissue samples were blotted and transferred to weighed vials for reweighing and measurement of radioactivity using an automated γ-counter (Wallac Wizard 3″, Perkin–Elmer Life Sciences, Boston, USA). Radioactivity was expressed as cpm (g wet tissue)$^{-1}$.

In a previous study the tissue uptake of radioactivity 20 min after injection of 2-[^{125}I]I-ICI-H (Scheme 1) increased linearly with injected dose and predosing with 2-I-ICI-H **1b** had no effect. It was assumed that radiolabelled acids (**11b**–**c** and **15b**–**c**) would be comparable. Therefore, to correct for differences in animal body weight and injected dose, results were expressed as an uptake index, defined as

$$\text{Uptake index} = \frac{\text{Tissue radioactivity (cpm)/tissue wet weight (g)}}{\text{Radioactivity injected (cpm)/body weight (g)}}.$$

Radioactive metabolites in plasma were assessed by gradient RP-HPLC as described above. Rats (250–320 g) were anaesthetized for insertion of catheters into the ventral tail artery and one lateral tail vein and were allowed to recover from the anaesthesia for ~2–3 h before injection of 37 MBq **15c** via the tail vein catheters. Blood (1 mL) was collected from the tail artery catheter at 2 or 25 min after injection of radioligand. It was spiked with 45 μg nonradioactive **15a**. Cell-free plasma was prepared by centrifuging each blood sample at 2000*g* for 2 min. Ice-cold acetonitrile (0.7 mL) was added to the plasma (0.5 mL) and the precipitated proteins were removed by centrifugation. 200 μL of the resulting spiked supernatant was injected on to the HPLC column (HPLC-conditions: see above). To evaluate the method 1.4 MBq **15c** were added to 1 mL blood in vitro and processed as described above.

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